capable of reacting with ligand (L) to the total amount of receptor (R1), thereby permitting the analyte concentration in the sample to be calculated.

The term "receptor" as used herein refers to active analyte-binding receptor, and, where relevant, active ligand-binding receptor, respectively, and is not meant to include such receptor in an inactive or non-binding state. Likewise, the term receptor-binding ligand refers to active receptor-binding ligand and is not meant to include such ligand in an inactive or non-binding state.

The term "amount" as used herein usually means binding capacity. Thus, for example, when it is stated that the amount of analyte-specific receptor is in excess of the amount of analyte, it means that there is more analyte-specific receptor than necessary to bind all analyte. Usually, there is a 1:1 reaction ration between e.g. the analyte and the analyte-specific receptor, or between the analyte-specific receptor and the immobilized receptor-binding ligand. In such a case, the binding capacities of the respective species correspond to their molar amounts. Other reaction ratios are, however, also possible. For example, the immobilized ligand may be capable of binding more than one analyte-specific receptor.

In another embodiment of method of the invention, the sample is contacted with analyte-specific receptor (R1) provided both in solution and, in a minor fraction, immobilized to a solid phase, thereby permitting a minor fraction of analyte present in the sample to be bound to the solid phase. If the ratio of the amount of receptor (R1) in solution to the amount of immobilized receptor (R1) is known, the analyte concentration in the sample may be calculated from the detected amount of analyte bound to the solid phase.

It is readily seen that the above procedure gives the same effect as diluting the sample. In addition to the dilution step being avoided, which, of course, is of advantage to the operator, one obtains a considerable saving in reagents, i.e. both the reagent for capturing the analyte on the solid phase and the detecting agent, the latter often being costly. In this connection, it is also to be noted that in the assay of the invention, the reaction between analyte and receptor takes place in solution where almost all receptors are active rather than at a solid phase surface as in a corresponding conventional assay where only about 10-20% of immobilized receptor will react (Butler, J. E., et al, Molecular Immunology, Vol. 30, No. 13, pp. 1165-1175, 1993).

The required ratio between the total binding capacity of analyte-specific receptor contacted with the sample and (i) the binding capacity of receptor-binding ligand that is immobilized to the solid phase when this is limited, or (ii) the ligand-binding capacity of the analyte-specific receptor when this is limited, is readily determined by the skilled person depending *inter alia* on the particular analyte to be determined and the particular assay format used and may be chosen within wide limits. Usually, this ratio is from about 2:1 to about 1000:1, especially from about 5:1 to about 100:1, preferably more than about 10:1, more preferably more than about 20:1.

The excess of analyte receptor relative to the amount of analyte in the sample is also readily determined by the skilled person for each specific case.

The receptor contacted with the sample is usually of the dual receptor or bireactive binder type having one part that specifically binds to the analyte and another part which specifically binds to the ligand immobilized on the solid phase surface. The analyte binding part may, for example, be an antibody (monoclonal or polyclonal) or an active fragment thereof (including recombinant antibodies and fragments) or nucleic acids whereas the ligand-binding part may be one member of a specific binding pair. Exemplary such specific binding pairs include immunological binding pairs, such as antigen-antibody and hapten-antibody, biotin-avidin or –streptavidin, lectin-sugar, hormone-hormone receptor, and nucleic acid duplex. For example, the solid phase may have streptavidin immobilized thereto, and the receptor for the analyte may be biotinylated. To avoid immunoprecipitation at high analyte concentrations, it may be preferable to use monovalent receptors.

While the analyte preferably is a molecule present in concentrations >1 nmole/litre in a sample, the analyte may, of course, be any substance for which there exists a naturally occurring analyte-specific binding member or for which an analyte-specific binding member can be prepared.

Analyte that has been captured by the solid phase is usually detected by reaction with a labelled specific binder for the analyte. Such a labelled binder may be a conjugate comprising a detectable label covalently or non-covalently attached to the specific binding member, "label" referring to any substance which is capable of producing a signal that is detectable by visual or instrumental means, particularly a fluorophore or chromophore.

The sample is usually of biological origin, for example blood (serum, plasma, whole blood), saliva, tear fluid, urine, cerebrospinal fluid, sweat, etc. The invention is, of course, also applicable to other types of samples, such as fermentation solutions, reaction mixtures, etc. Especially, however, the sample is an undiluted serum or whole blood sample.

While the present invention is generally applicable, it may advantageously be used in solid phase assays of the immunochromatograpic type. Such assays use a device comprising a plate-shaped flow matrix of bibulous material, usually a membrane strip, such as of cellulose nitrate or glass fiber, in which liquid can be transported laterally (i.e. in the plane of the strip) by capillary forces in the membrane. The membrane usually has a sample application zone, and a detection (or reaction) zone downstream of the sample application zone. In the detection zone, usually a capturing reagent for the analyte is immobilized. To conduct an assay, the application zone is contacted with the liquid sample to be assayed for the analyte of interest. The device is maintained under conditions sufficient to allow capillary action of liquid to transport the analyte of interest, if present in the sample, through the membrane strip to the detection zone where the analyte is captured. The capillary liquid flow is usually insured by an absorbing pad or the like at the downstream end of the strip. A detection reagent, usually labelled, is then added upstream of the detection zone and interacts with captured analyte in the detection zone, and the amount of captured analyte is measured. Often, the detection reagent is pre-deposited in or on the membrane strip, e.g. in the form of diffusively movable particles containing fluorophoric or chromogenic groups, either upstream of the sample application zone or between the sample application zone and the detection zone.

In an immunochromatographic assay according to the invention, the receptor is added to the sample either before applying the sample to the membrane strip, or may be pre-deposited in or on the membrane strip upstream of the detection zone.

A test kit for carrying out the method of the invention may comprise such a membrane having (i) immobilized in or on the membrane a ligand which binds specifically to the receptor, and (ii) dissolvably pre-deposited in or on the membrane a specified amount of analyte-specific receptor. The amount of the ligand on the solid phase member is less, and usually considerably less than that required to bind the specified amount of the receptor substance.